

Dengue Hemorrhagic Fever at 60 Years: Early Evolution of Concepts of Causation and Treatment

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SUMMARY

During the decade of the 1960s, the epidemiology of a new dengue disease, dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS), was described by collaborative research performed by Thai scientists from many institutions and by workers at the U.S. Army's SEATO Medical Research Laboratory in Bangkok, Thailand. Careful clinical and physiological studies provided the initial description of DSS. DSS cases were caused by each of the four dengue viruses (DENV) and not chikungunya (CHIK) virus or DENV 5 and 6, were associated with a secondary-type dengue antibody response in children over the age of 1 year, were associated with a primary antibody response in infants less than 1 year old whose mothers had neutralizing antibodies to all four DENV, were associated more frequently with secondary DENV 2 infections than those due to DENV 1 and 3, and were more common in females than males over the age of 3 years. Robust laboratory methods for growth and recovery of DENV in tissue cultures were introduced. In addition, life-saving principles of fluid and plasma protein resuscitation of hypovolemia were described. Most epidemiological observations made during the decade of the 1960s have been confirmed in the succeeding 45 years. Much contemporary research on pathogenesis fails to address the two distinct immunological antecedents of DHF/DSS.

INTRODUCTION

Dengue hemorrhagic fever (DHF), an integral component of the dengue pandemic of the 20th to 21st centuries, emerged a little more than 60 years ago. In September 1954, Quintos et al. described 21 cases of a severe febrile disease of children living in or

near Manila, characterized by fever, flushed face, abdominal pain, positive tourniquet test, thrombocytopenia, narrow pulse pressure, shock, gastrointestinal hemorrhages, depressed bone marrow, and a high case fatality rate (1). Similarities in presentations between these cases and those of "epidemic hemorrhagic fever" (now called hemorrhagic fever with renal syndrome), then a well-recognized acute disease among combatants fighting in Korea, impelled the authors to identify this entity as a "hemorrhagic fever" (1). However, recognizing the absence of a renal component, the authors soon changed the name to "Philippine hemorrhagic fever" (PHF) (2). During the rainy season of 1956, an additional 1,200 cases of PHF occurred. By chance, William M. Hammon, Director, Commission of Virus and Rickettsial Diseases, U.S. Armed Forces Epidemiology Board, was in the Philippines that year to study the distribution of vector-borne viral infections. He quickly identified dengue viruses (DENV) as the etiology of PHF, with most cases being attributed to two new viruses, DENV 3 and 4 (3, 4).

In 1958, an outbreak of 3,500 cases of Thai hemorrhagic fever (THF) resulted in an invitation to Hammon and his team

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to study the disease in Bangkok. During this visit, chikungunya (CHIK) virus and DENV of multiple types were recovered from clinical cases and *Aedes aegypti* mosquitoes. The DENV isolated were designated DENV 5 and 6 (5, 6). PHF and THF were quite puzzling, as the clinical course bore little resemblance to that of classical dengue fever (DF), a debilitating but nonfatal febrile exanthem.

In September 1961, 7 years after the description of PHF by Quintos et al., one of us (S.B.H.), a commissioned officer in the U.S. Army Medical Corps, was assigned to establish a research program on dengue in Bangkok, Thailand, at the Southeast Asia Treaty Organization (SEATO) Medical Research Laboratory (SMRL). Through the collaboration of the Faculty of Public Health of the University of Health Sciences, the generous support of the U.S. Army, and the help of a virtual army of scientific colleagues, a large multidisciplinary research program was initiated. While SMRL, now designated the Armed Forces Research Institute of the Medical Sciences (AFRIMS), operates to this day as a global leader in dengue research, this review focuses only on the results and concepts that emerged during the first decade of studies on the clinical, epidemiological, and pathogenic aspects of human dengue that prepared the ground for successive research discoveries that underlie concepts held today.

In September 1961, what was known about dengue? Early in the 20th century, the DF syndrome was demonstrated to be caused by a virus and transmitted by *Aedes aegypti* (7–9). In follow-up studies, the clinical and laboratory responses to DENV infection of adults were fully described in a human infection model in Australia, the United States, Philippines, and Netherlands (through infected mosquitoes from Indonesia) (9–13). Based on the well-known clinical presentation and epidemiological features of DF described in published reports, outbreaks had been reported from around the globe beginning in the 18th century. During World War II, pan-Pacific outbreaks of DF, particularly among combatants, resulted in the recovery of DENV 1 in Japan, Hawaii, and India and of DENV 2 in New Guinea (14, 15). A decade later a DENV 2 strain was isolated from human cases of DF on Trinidad Island in the Caribbean (16). Against this large historical experience, it came as a surprise that DENV were associated with a fatal disease in Southeast Asian children exhibiting almost none of the clinical features of DF.

Just prior to the establishment of studies on THF in September 1961, W. M. Hammon's team and an interdisciplinary group of Thai workers presented recent research findings at a symposium on hemorrhagic fever held in Bangkok on 11 to 12 August 1961 (17). These and other studies are summarized in Table 1. The results included the recovery of 6 types of DENV from PHF and THF cases. Adding to this complexity, chikungunya (CHIK) viruses were also recovered from THF patients, but it had not been possible to differentiate the illnesses caused by these two taxonomically distinct viruses. Among children admitted to hospital in shock, mortality rates were as high as 24% (18). Aspirin was found to increase the mortality rate for THF. Many physicians, confronting such high mortality rates, used high doses of hydrocortisone to treat shock. Two additional "facts" about Thai hemorrhagic fever were known: (i) infections by either DENV or CHIK virus resulted in severe and fatal disease in indigenous children, but foreign residents of Thailand, when infected with the same viruses, experienced only DF (19), and (ii) pediatricians in a

major Bangkok hospital thought that THF might be due to "Chinese medicine poisoning" (19).

To unravel these complexities a research collaboration was established with the Department of Microbiology (Chair, Charas Yamarat), Thailand Faculty of Public Health (FPH), with Suchitra Nimmannitya and other physicians at Bangkok Children's Hospital (BCH), and with John Scanlon, Head, Entomology Department, SMRL. The SMRL Virology Department itself was sited in an FPH laboratory constructed with Rockefeller Foundation funds. The FPH was located across the street from SMRL headquarters and immediately adjacent to the BCH. Six longitudinal studies were initiated: (i) at the BCH, etiological, clinical, and hematological studies of febrile outpatients, in-patients with an admission diagnosis of, THF and controls admitted for surgical procedures during 1962 to 1965 (20–22); (ii) a special study on the pathophysiology and treatment options for hospitalized THF patients was established during 1964 (23–25); (iii) etiological and clinical studies of foreign residents of Bangkok at the U.S. Embassy-Joint U.S. Military Advisory Group Medical Unit, at three schools and two hospitals catering to foreign residents of Bangkok, were conducted in 1962 and 1963 (26); (iv) a prospective seroepidemiological cohort study was established in 19 randomly selected locations in Bangkok to measure DENV transmission and disease occurrence among 48,401 enrolled residents during 1962 to 1964, (27); (v) a study to identify vector mosquitoes of DENV and CHIK virus was established using light- and human-baited traps in 5 locations in Bangkok in 1962 to 1964 (27, 28); and (vi) data on hospital admissions for THF were collected annually from all 21 Bangkok and Thonburi hospitals and 101 provincial and private hospitals outside Bangkok in Thailand in 1962 to 1964 (29). These and companion studies yielded a steady stream of data that fundamentally changed the understanding as well as the management of dengue problems.

SOLVING TECHNICAL PROBLEMS: IMPROVED LABORATORY METHODS

The first challenge to dengue research in Thailand was how to increase the efficiency of recovering dengue viruses for laboratory study. At the time, all laboratory strains of dengue viruses had been recovered by intracerebral (i.c.) inoculation of young mice. It had taken 10 to 20 blind passages to recover DENV from patients' blood or mosquitoes captured in the Philippines and Thailand (4, 30). At the SMRL, DENV recovery was initially approached using blind passage in suckling mice. All suspect materials were inoculated at 0.01 ml i.c. and 0.02 ml intraperitoneally in 1-day-old Swiss Webster white mice and then routinely blind passaged twice at 10 days to the third passage (20). Outside Thailand, many laboratories were pioneering in the use of tissue cultures to recover other wild-type viruses from infected humans and for the preparation of virus seeds. In 1962, the SMRL succeeded in preparing DENV seed viruses in cell cultures by adapting the challenge virus system pioneered in the discovery of rubella virus (31). Primary monkey kidney and continuous hamster kidney cells, BSC-1, LLC-MK2, PS, and HeLa were tested as assay systems and used to prepare seed viruses from mouse-adapted strains of DENV 1, 2, 3, 4, TH-36, and TH-Sman. Some DENV, at high concentrations, produced a cytopathic effect (CPE). At low concentrations, none produced CPE, while infected cells resisted challenge with CPE-producing strains of poliovirus type 1, chikungunya virus, or coxsackie B1 virus. BSC-1 cells were found to be

TABLE 1 Observations on clinical findings, epidemiology, etiology, and treatment of dengue hemorrhagic fever cases, 1954 to 1961

Category	Findings	Reference(s)
Clinical	In Thailand, cases of “influenza with secondary thrombocytopenic purpura” or “influenza with circulatory failure” were seen on the Siriraj Hospital pediatric ward each year from 1951. In these cases, toxic shock or hemorrhagic stage begins on days 3 to 7 after fever onset, usually coinciding with fall in temp. 64% had thrombocytopenia. Shock was characterized by narrow pulse pressure and elevated hematocrit.	18
	In PHF, common clinical findings were positive tourniquet test, thrombocytopenia, narrow pulse pressure, and gastrointestinal hemorrhages, with a high fatality rate in shock cases.	1, 79
	Chest X rays showed pneumonic infiltrates and right-sided pleural effusion.	19
	In THF, liver was enlarged in 92% and tourniquet test positive in 87.5% of cases. In PHF, no liver enlargement was described.	48
	In 1958, grades III and IV ^a comprised 39.5% of admissions, and there was 78% case fatality in grade IV.	48
	Bone marrow shows maturation arrest of megakaryocytes.	19
Epidemiology	For THF, in 1958 to 1960, infants less than 1 yr old comprised the largest group of cases and deaths.	19, 48
	There were biannual epidemics during the rainy season. Modal ages of admission were 3 yr in 1958 and 5 yr in 1960. Overall, the case fatality rate was 12.25%.	50
	There were more deaths in females than in males.	50
	THF occurred in nearby provinces, but case fatality rates for children coming from cities outside Bangkok were higher than those among Bangkok residents.	50
	Chinese children are “more susceptible” to THF than are Thai children (a higher proportion of THF cases were Chinese than in hospital for other conditions).	19, 50
	DF occurred in adult foreign residents; THF was seen in indigenous children.	104
	<i>Aedes aegypti</i> distribution geographically correlated with THF cases; <i>A. albopictus</i> did not.	52, 105, 106
Etiology	There were autopsy reports on 63 cases, 1957 to 1960. Six were infants less than 1 yr old. All THF cases had peripheral vascular collapse prior to death. There were interstitial hemorrhages in many organs and bleeding by diapedesis. There were serosal effusions (pleural > peritoneal > pericardial). Damage to endothelial cells was not visible.	107
	Complement fixation and mouse i.c. neutralization studies on DENV using mouse and monkey DENV antisera revealed 6 types. DENV 1 and DENV TH-Sman (DENV 5) and DENV 2 and TH-36 (DENV 6) are closely related.	5
	DENV 2, DENV 4, and chikungunya virus were isolated from THF nonshock cases.	5
	Simultaneous chikungunya virus and DENV antibody responses were identified in severe THF cases.	30
	DENV 4 was recovered from liver tissue from a fatal case of THF.	108
	Symptoms and signs in cases with dengue virus or chikungunya virus isolations could not be differentiated.	49
Treatment ^b	For shock cases, glucose in water, antibiotics, and cortisone were given.	19
	Children given aspirin prior to admission had higher fatality rates than children not given aspirin.	48
	Shock patients were given ephedrine sulfate and blood transfusions (to expand blood vol).	2
	When treatment was ineffective, children usually died within 12–24 h of admission. When it was effective, children made an uneventful recovery.	48

^a Children's Hospital clinical classification: grade I, high fever, with no hemorrhagic manifestations other than positive tourniquet test; grade II, fever, hemorrhagic signs, or rash, with no peripheral circulatory failure; grade III, fever, signs of peripheral circulatory failure, cool extremities, circumoral cyanosis, profuse sweating; grade IV, fever, profound shock, cold, clammy, and congested extremities, no pulse or blood pressure (48).

^b “On the question of treatment, we should attribute the main benefit to the use of hydrocortisone in all hospitals in Bangkok”—Svasti Skulthai, Chulalongkorn and Siriraj Hospitals (109).

sensitive to strains of all DENV at the same titers as in suckling mouse i.c. assays (32). It quickly proved possible to recover wild-type DENV in BSC-1 using poliovirus type 1 challenge. In an initial study, strains of DENV 1, 2, and 3 were recovered from 18 acute-phase sera and 10 pools of wild-caught *Aedes aegypti* (33). From mid-1963, recovery of DENV in tissue culture became a routine. During 1962 to 1965, 145 DENV 1 to 4 viruses were recovered in BSC-1 cells, 140 in suckling mice, and 23 in both recovery systems (34). Using this technical advance, a DENV neutralization test was developed in BSC-1 cells (32). Finally, with patience and perseverance, a robust agar overlay DENV plaque assay was developed using LLC-MK2 cells (35). This plaque assay was adapted to the plaque reduction neutralization test (PRNT) still in use today at AFRIMS and worldwide (36, 37).

Once the recovery of wild-type DENV had been simplified,

reliable identification and classification of DENV were imperative. Hammon et al. had relied upon the complement fixation (CF) or mouse i.c. neutralization test. At the time, typing sera were prepared using multiple inoculations of DENV mouse brain suspensions in mice, hamsters, guinea pigs, rabbits, or monkeys. CF and neutralizing antibodies prepared in immunized mice or DENV-infected monkeys were successful in distinguishing DENV 3 and 4 from DENV 1 and 2 (38). However, the same test systems found evidence for the existence of DENV 5 and 6. It was observed that DENV 1 and DENV TH-Sman (DENV 5) and DENV 2 and TH-36 (DENV 6) were closely related using CF tests of viral antigens with mouse sera. Sera recovered from infected humans often reacted with either DENV 5 or 6 more strongly than they did with prototype DENV 2 or 1, respectively (38). Over the next few years, the introduction of tissue culture neutralization tests and, partic-

ularly, the preparation of antisera in monkeys infected with a single dose of DENV made it possible to demonstrate that TH-Sman was a strain of DENV 1 and TH-36 a strain of DENV 2 (34, 39). There were only four DENV!

EVOLUTION OF CONCEPTS OF CAUSATION

Etiology, Dengue versus Chikungunya: the Dengue Shock Syndrome

Important contradictions in data and hypotheses on the causation of PHF and THF required attention. Why were 8% of cases caused by CHIK virus and another 3% caused by mixed DENV and CHIK virus infections (20)? CHIK mild febrile disease in children was often confused with DENV infection. In Bangkok, DENV and CHIK virus accounted for a significant fraction of all outpatient visits; 8% were caused by CHIK virus and 29% by DENV (21). CHIK virus seemed to be an important component of severe disease, as suggested by the introduction of CHIK into Kolkata in 1963 from East Africa, which was followed by a nationwide epidemic that included severe and fatal cases of a hemorrhagic disease in children. However, it soon was established that DENV were also involved (40).

The data that resolved these contradictions were developed following a visit to Bangkok in mid-1963 by one of us (S.N.C.), a Johns Hopkins' trained pediatrician, assigned to the Department of Biochemistry, Walter Reed Army Institute of Research. Based upon his review of THF cases with clinicians, S.N.C. was alarmed at high case fatality rates. A plan was made to establish a Thai Hemorrhagic Fever Study Center (THFSC) for the purpose of better understanding the pathophysiology of the THF and evaluating therapeutic regimens for serious cases. The Ministry of Public Health of Thailand authorized the creation of the center in 1964, granting permission for S.N.C. to serve as its clinical director. The Thai Army Pramongutklo Hospital furnished clinical and laboratory facilities, WRAIR supplied pharmaceuticals, intravenous fluids, commercially available human plasma proteins, a variety of surgical instruments, and resuscitation equipment, and SMRL provided the logistical and financial support for round-the-clock clinical and laboratory services. Between 4 July and mid-October 1964, the THFSC admitted 149 patients, ages 5 months to 17 years, among whom were 126 with DENV and 5 with CHIK infections. The center provided the first description of the dengue shock syndrome (DSS), manifested as a narrow pulse pressure (<20 mm Hg), associated with hemoconcentration [(highest hematocrit – recovery hematocrit)/recovery hematocrit ≥ 0.2] and, idiosyncratically, hypoproteinemia (23, 25). These observations implied the loss of both fluid and protein from the circulation into serosal spaces, a type of viral pathophysiology never previously described. Only DENV etiology was associated with DSS in THFSC patients and also when the new case definitions were applied to the larger group of patients admitted to the BCH study (20). DENV and CHIK illnesses differed in other ways. A non-shock illness with petechial rash or a febrile illness with a duration of greater than 6 days with mild hemorrhagic manifestations was likely to be of DENV origin, while patients with mild hemorrhagic manifestations, arthralgia, conjunctival injection, and a fever of sudden onset with a duration of only 2 to 3 days were likely to have CHIK infections (42).

Host Susceptibility

Primary versus secondary DENV infection. Why did DENV and CHIK infections in foreign residents, mostly adults (26), result in DF while indigenous children infected with the same viruses developed THF (20)? Critical to answering this question was a careful definition of clinical syndromes. The provisional definitions used by S.B.H. to analyze BCH and THSC data were as follows: for DF, a mild, self-limited febrile illness with serological or virological evidence of an acute dengue infection but no specified signs or symptoms; for DHF, a dengue disease characterized by worsening of illness two or more days after onset of fever, with hypoproteinemia (≤ 5.5 g%) and one or more of the hemostatic abnormalities thrombocytopenia ($<100,000/\text{mm}^3$), prolonged bleeding time (>5 min), or elevated prothrombin time; for DSS, a useful diagnostic and prognostic subgroup, a dengue disease characterized by the above plus shock (hypotension or pulse pressure of ≤ 20 mm Hg) and hemoconcentration as evidenced by a hematocrit of $\geq 20\%$ of the convalescent-phase value, usually with an increase in serum transaminases (43). Applying the case definition of DSS to the entire group of BCH and THFSC patients, a secondary-type dengue antibody response was found to be a critical risk factor. Of 196 patients in shock, 186 experienced a secondary DENV infection, and most of the 10 with primary infections were infants (see below) (44). A second, third, or fourth DENV infection would raise an anamnestic antibody response. Which of these infection sequences resulted in DSS? A study of the transmission dynamics of four different DENV using a mathematical model showed the age distribution of cases admitted to Thai hospitals to be consistent only with a second, not a third or a fourth, DENV infection (45).

Caucasians versus Asians. As a result of the findings described above, the etiological landscape had changed irretrievably. DSS required sequential infection with two different DENV—the “two-infection” hypothesis. It was now clear why DSS did not occur in foreign residents of Bangkok. Sequential DENV infections in this group were very rare. Indeed, based upon a pre- and post-rainy season serological survey in 1962, of 395 children and adults, only 1.3% of foreign residents experienced a DENV infection, compared with a dengue infection rate of 41% that year among indigenous residents of Bangkok (26, 27). The two-infection hypothesis of DSS explained the occurrence of DF in foreigners without resort to the assumption that racial differences influenced response to infection. Occasionally, however, two infections did occur in foreign residents of Thailand, with at least one documented as classical DSS (46).

Among the estimated 47,000 foreign residents of Bangkok in 1962 to 1964, 11 illnesses caused by CHIK virus were documented. None of these patients had an illness characterized by shock. All had dengue-like symptoms, and half had postillness arthralgia. The clinical diagnosis “dengue” was often given to foreign residents of Bangkok seeking medical counsel for febrile illnesses, but only during the rainy season were DENV or CHIK infections documented serologically (26). It was also established that CHIK infections in indigenous Bangkok children contributed to the large group of mild cases in the THF patient population but not to those with DSS or severe hemorrhages (20, 21).

Chinese versus Thai. Of 471 DENV-infected children admitted to BCH or THFSC during 1962 to 1964, 133 (28%) were Chinese. In contrast, of the 31 children hospitalized for THF of CHIK

etiology, only 9.6% were Chinese (20). Over this same period, in the outpatient department of Children's Hospital, 85.1% of patients were Thai and 14.9% Chinese (21). To explain the greater representation of Chinese children admitted to hospitals for DHF/DSS in contrast to the ratio of Thai to Chinese children with mild illnesses caused by either DENV or CHIK virus, it was concluded from the testimony of parents in the large prospective Bangkok Area Study that Chinese parents recognized the early life-threatening signs and symptoms of DHF/DSS and sought modern Western medical care in Bangkok hospitals. For milder and non-life-threatening illnesses, Chinese parents sought more traditional kinds of health care (27). In other words, Chinese and Thai children were admitted to hospital for DHF/DSS in accordance with their composition of the Bangkok population. Chinese children were not at greater risk to DHF/DSS than Thai children.

Sex. Females predominated among patients hospitalized with THF and dengue shock syndrome during the period 1958 to 1964 (20, 48, 49) and among children dying of THF during the period 1958 to 1964 (50, 51). In 1958, among THF hospitalizations there were twice as many deaths in females than in males, a phenomenon almost unheard of among infectious diseases. Overall, in the BCH/THFSC series there were more female than male patients with a laboratory diagnosis of dengue infection, with a strongest trend among children with a secondary DENV infection. It was notable that in the shock group there were twice as many girls as boys. However, the predominance of females over males began only in children over the age of 3 years (42). There was no parallel increase in antibody response to DENV infection by sex in this series (42).

Immune status. The puzzle of different clinical syndromes in indigenous children versus Caucasian foreign residents attracted many hypotheses. Was severe disease in children the result of simultaneous infection with two DENV or with DENV and CHIK virus, or might it be due to nutritional or genetic differences between these hosts (52–56)? Although there were cases of DHF with primary immune responses in the BCH and THFSC series (29), secondary-type DENV antibody responses were consistently and highly significantly associated with DSS (20, 42, 44). The criteria differentiating primary and secondary DENV antibody responses had been newly rooted in modern IgM and IgG kinetics (57, 58). In addition, there were two strong serological criteria that could differentiate primary from secondary antibody responses. First, in the absence of a 4-fold or greater rise in titer in acute- and convalescent-phase samples, a convalescent-phase hemagglutination inhibition (HI) DENV titer of 1:640 was accepted as evidence of a recent secondary dengue infection. This value was shown to be statistically significantly higher than mean HI values in 1,878 Bangkok residents of all ages who were sampled at the end of the rainy season in 1962 (27). Second, it was observed that only IgG DENV antibodies fixed complement. For this reason, the detection of DENV CF antibodies in acute- or early-convalescent-phase sera provided evidence of a secondary DENV antibody response (44, 57, 58).

Viral Virulence

By 1963, many dengue workers asked whether dengue viruses with serious pathogenic potential were at loose in the world (3). In an attempt to answer this question, historic records of the Pediatric Service at Siriraj Hospital and Medical School were reviewed.

From a chart review, children with a disease consistent with DHF had been hospitalized every year beginning in 1950. Unfortunately, for the years 1943 to 1948, wartime hospital charts were missing. Fortunately, immediately prior to World War II, patient charts were recorded in English under the supervision of American medical faculty members sponsored by the Rockefeller Foundation. A review of 572 of these charts selected randomly from admissions in July and August, 1932 to 1942, failed to identify any cases consistent with the DHF syndrome (55). It appeared that “virulent” DENVs appeared after World War II. But did they? In the medical literature, dengue-like outbreaks with high case fatality rates had been reported in Queensland, Australia (1897), the southern United States (1922), Durban, South Africa (1927), Athens, Greece (1928), and Taiwan (1933). At least two of these outbreaks, in Queensland in 1897 and Athens in 1928, involved children and adults where a vast majority of adults experienced classical DF. It was further noted that severe disease accompanied the second of closely spaced consecutive DF outbreaks (54, 56, 59). But are DENV “virulent”? During 1962 to 1964, from patients with secondary-infection DHF and shock, DENV 2 was recovered 7 and 5 times more frequently than DENV 1 or 3, respectively (20, 43). Yet, during the same period and location, 26, 27, and 24 isolates of DENV 1, 2, and 3, respectively, were recovered from patients with primary DENV infections, and 6, 8, 8, and 2 isolates of DENV 1, 2, 3, and 4, respectively, were recovered from wild-caught *Aedes aegypti*. In other words, although the DENV 1, 2, and 3 forces of infection were identical over this period, secondary-infection sequences ending in DENV 2 occurred in DHF/DSS patients at high rates and were more severe than secondary-infection disease caused by DENV 1 or 3. In the laboratory, as a virus, DENV 2 seemed to be exceptional. Compared with other DENV, it produced higher-titer viremias in monkeys and higher growth in cell cultures and cross-reacted more frequently with antisera to other DENV. Perhaps DENV 2 was more “virulent” than other DENV?

Chinese Medicine Poisoning

At Bangkok Christian Hospital in 1958, “not a single case of THF was diagnosed among either Americans or Indians,” while 23% were in Thai children and 77% in Chinese children (19). During this same period, the ethnic distribution among hospitalizations for all conditions was 57% Chinese and 38% Thai. The author went on, “In our hospital we had seen this bizarre clinical pattern, with almost all of the patients dying rapidly in a shock-like state and responding poorly to supportive therapy. . . When we delved into the history of these children, we find all had been given Chinese medicine, usually for fever. . . We had previously incriminated the Chinese medicine as the cause of death” (19). Toxicity seemed plausible in a child with the onset of high fever followed in several days by restlessness, nausea, vomiting, abdominal pain, and cool extremities, delays in seeking medical assistance, and irreversible shock. With subsequent experience, it became clear that the time of admission after onset of fever was a variable of prognostic importance. A delay in medical interventions could lead to irreversible shock. During the early 1960s, there was a steep learning curve among parents and physicians in Bangkok. Earlier hospital admissions led to falling case fatality rates and the disappearance of “Chinese medicine poisoning.”

Primary-Infection DHF in Infants: Was It Real?

By the late 1960s, the “two-infection” hypothesis had been confirmed in children who were followed prospectively (60–62). This led to a hypothesis that IgG-DENV immune complexes mediated by activated complement were pathogenic (58, 63). In studies to confirm to this hypothesis, performed largely on children with secondary DENV immune responses, significant activation of the complement system during DHF/DSS was documented (64–66). However, some children admitted to BCH had DHF/DSS accompanied by a primary DENV antibody response. These children were younger than others with secondary antibody responses, who at that time were distributed around a mode of 4 years (20). Two children, ages 4 and 9 years old, with primary DENV infections had DSS. However, the largest group with primary antibody responses were infants less than 1 year. If these cases conformed to clinical and physiological criteria for DSS, the “two-infection” hypothesis of DSS immunopathogenesis was not sustainable. In 1966, S.B.H. returned to Bangkok expressly to determine if infants had been clinically misclassified. There, he found that Kamolwat Vini-chaikul, pathologist at Women’s and Children’s Hospital, had in his files autopsy and laboratory records and clinical chart summaries from more than 200 infants and children who had died of DHF/DSS during 1958 to 1965. Among those were 35 complete autopsies on infants less than 1 year old. On careful inspection, the clinical course, acute-phase laboratory results, and gross and microscopic pathological findings in infants were found to be identical to those for DHF/DSS in children over the age of 1 year (25, 42, 43). Subsequently, the identity of the pathological findings in 6 infants with those of 94 older children was affirmed in a classic study (67). Of three infants included in a special hematology study, all had a positive tourniquet test and 2 were in shock with thrombocytopenia, prolonged bleeding time, and elevated silicone clotting time (22). In addition, DENV 2 had been recovered and low levels of C3 and C4 detected in acute-phase serum from a 6-month-old female admitted with DSS in a 1971 study. The infant’s mother had neutralizing antibodies to all four DENV (66). The conclusion was that infant DSS accompanied by primary DENV infections had all the features of DSS in children with secondary DENV infections.

HEMORRHAGIC FEVER: IS IT PROPERLY NAMED?

In the decades after the 1960s, as DENV transmission spread around the world, a substantial percentage of patients, hospitalized and nonhospitalized, suspected of having DHF/DSS with documented DENV infections failed to display hemorrhagic phenomena, including a positive tourniquet test (68–74). Indeed, as a result of these observations, a WHO committee recommended that the 1997 WHO case definition of DHF/DSS be abandoned in favor of “severe dengue” (75). In part, the decreased rate of severe hemorrhage accompanying DENV infections with the passage of time was real. As parents and physicians appreciated that DENV infections could be life-threatening, patients sought care earlier in the course of illness. Prompt and improved fluid resuscitation not only decreased case fatality rates but reduced the incidence of gastrointestinal hemorrhage, usually a complication of prolonged shock (76). It is now acknowledged that endothelial damage during DENV infections results in increased capillary permeability more often than in overt hemorrhage (77, 78). However, from the

perspective of early observations on PHF and THF cases, severe bone marrow depression, maturation arrest of megakaryocytes, severe thrombocytopenia, and prolonged bleeding time had been repeatedly documented with and without accompanying severe hemorrhaging (Table 1) (1, 2, 79, 80). A special hematology study of 27 THF patients in 1962 included 9 children with DSS. Seven had secondary DENV antibody responses, while 2 were infants less than 1 year old with primary DENV antibody responses. Six of these patients had thrombocytopenia, positive tourniquet tests, prolonged bleeding times, and prolonged silicone clotting times, and accompanied by elevated serum glutamic oxaloacetic transaminase (SGOT) levels. Four had elevated prothrombin times. One-third of these cases had no hemorrhagic signs during the course of illness. Thus, altered hemostasis and liver damage are important components of the DENV-mediated vascular permeability syndrome, justifying the retention of the diagnostic term “hemorrhagic fever.”

TREATMENT OF DSS

Why were THF case fatality rates so alarmingly high? This question was confronted directly by the THFSC. A breakthrough in understanding of the cause of death in THF patients occurred when, during the postmortem examination of the first patient to succumb, fluid was noted in the thoracic and peritoneal spaces. Fluid reaccumulated (i.e., seeped from the tissues) as rapidly as it could be removed with gauze pads. It was clear from these observations that previously administered intravenous fluids had left the vascular compartment and had accumulated in the extracellular space and the (so-called) third space. Careful observation of the natural course of THF in later patients revealed that in some, serum albumin diminished during the administration of crystalloids (23, 25). These same patients developed a hemoconcentration of 20% or more compared with the convalescent-phase value as measured by microhematocrits at the bedside during this phase of the disease. If this was not corrected rapidly, patients developed shock, many with low pulse pressure (≤ 20 mm Hg) yet retaining an acceptable systolic value. Blood transfusions were contraindicated in this situation, since the patients are already hemoconcentrated. Rather, a serum protein preparation was administered in place of the crystalloid infusion until the hematocrit stabilized and/or began to fall. This alternation of crystalloid with serum protein solution based upon hematocrit level measured at the bedside is similar to the regimen developed to treat severe burn patients in the early stages of their treatment. It was noted that hypoproteinemia played a role in the development of hemoconcentration and shock and that serum protein levels could be used to predict the response to intravenous fluid repletion (23). The frequent assessment of hemoconcentration with hematocrit values using microhematocrit centrifuges stationed on the treatment ward was critical to timely and physiologically relevant management. Once the new regimen was implemented, patients who appeared to be developing an irreversible shock were resuscitated and maintained until their acute illness abated after 24 to 36 h (24, 25). Once they were able to maintain their hematocrits without intravenous treatment, patients recovered rapidly and could be discharged home with no sequelae. Indeed, the fatality rate among THFSC children was 3%, well below the rate elsewhere in Bangkok at the time (20). Based upon clinical assessments and accepted practice standards, there was no requirement for the use of pressor amines, alpha adrenergic blocking agents, aldosterone, steroids,

TABLE 2 1964 WHO seminar suggestions for further studies^a

Suggestion
1. Further research on improved methods for isolating dengue viruses is desirable—methods that are rapid, sensitive, and not likely to modify the character of the original virus population found in the invertebrate or vertebrate host. Plaque isolation in tissue culture deserves particular attention. Methods to dissociate virus from antibody in human specimens should be explored.
2. Improved methods of antigenic analysis for the typing of dengue virus deserve increased effort.
3. When adequate typing is established, it is exceedingly important to determine whether it is possible to differentiate viruses causing the severe hemorrhagic syndrome from those causing classical dengue.
4. Further study needs to be directed at determining whether host factors, such as immunological sensitization or dietary or genetic factors, play essential roles in determining susceptibility to the hemorrhagic syndrome.
5. Studies are needed to localize the tissues and organs in which virus multiplies in humans, to assist in explaining pathogenesis, and to indicate appropriate sources for virus isolation at autopsy.
6. Studies on the physiology of the shock and preshock stages must be extended to enable a more rational approach to the prophylaxis and therapy of this manifestation of the disease. This will require close cooperation between physiologists and clinical pharmacologists in carrying out well-designed, controlled tests of therapeutic methods, and the immediate initiation of such tests should be actively encouraged.
7. Classical dengue in areas free from hemorrhagic fever should be studied carefully by hematologists to determine similarities and dissimilarities related to hemopoietic tissues, platelets, and hemostasis. Capillary fragility tests and capillary biopsies may prove to be enlightening.
8. <i>Aedes albopictus</i> should be tested in the laboratory in parallel with <i>Aedes aegypti</i> for quantitative study of comparative transmission efficiency, with use being made of dengue viruses of several antigenic types isolated from true hemorrhagic fever cases.
9. <i>Culex tritaeniorhynchus</i> and <i>Culex gelidus</i> should be tested for ability to transmit chikungunya virus.
10. A variety of domestic and wild mammals and birds as well as amphibians and reptiles should be inoculated with chikungunya virus and tested for viremia and antibody response.
11. Continued studies in search of a zoonotic forest reservoir of dengue viruses are required. If viruses in a sylvan cycle are recovered, they should be compared with those of classical dengue and hemorrhagic fever.
12. Inactivated polyvalent dengue virus vaccines require further exploration, particularly from tissue culture sources.
13. The establishment of effective and long-lasting vector control measures in Southeast Asia and the Western Pacific should be preceded and accompanied by extensive research on <i>Aedes aegypti</i> and related species. This research should include confirmation of vector ability, vector distribution, bionomics, ecology, and insecticide susceptibility.
14. Attempts should be made to utilize the high titers of dengue and chikungunya virus antigens present in acute-phase sera in an immediate <i>in vitro</i> diagnostic test.
15. Suitable diagnostic criteria and a classification and nomenclature based on symptoms, physical examinations, and clinical laboratory findings (including hematology and blood chemistry) should be developed that would be applicable to all variations of the diseases caused by dengue and chikungunya viruses, as seen in the different countries where these are etiological agents of hemorrhagic fever. A suggested system is presented in the annex to this memorandum.
16. There is a need for continued—and, indeed, increased—cooperation among the various research and clinical groups and individuals engaged in work on hemorrhagic fever and within and between cities and countries. Communication of an informal nature and through organized conferences, small and large, can be expected to contribute significantly toward these goals.

^a Reprinted from reference 83 with permission of the publisher.

platelet-rich concentrates, or routine blood transfusions (25, 59). Such treatments were then in wide use in Thailand and elsewhere in Southeast Asia (2, 18).

WHO INTERREGIONAL SEMINAR ON MOSQUITO-BORNE HEMORRHAGIC FEVERS IN THE SOUTHEAST ASIA AND WESTERN PACIFIC REGIONS, 19 TO 26 OCTOBER 1964, BANGKOK, THAILAND

Staff from Headquarters and the Southeast Asia and Western Pacific Regional Offices of the World Health Organization, international experts, and Thai and U.S. dengue scientists organized an interregional seminar on mosquito-borne hemorrhagic fevers, which was held on 19 to 26 October 1964 in Bangkok. The meeting was attended by 120 clinicians, hematologists, epidemiologists, pathologists, virologists, and entomologists from India, Singapore, Malaya, Thailand, South Vietnam, Philippines, Australia, Japan, the former Soviet Union, Europe, and the United States. Full-length manuscripts from 63 scientific presentations were published as full-length abstracts in the Bulletin of the World Health Organization (81). To this day, this is the largest and broadest compilation of studies and contemporary reports on dengue, chikungunya, and DHF/DSS in published form. Accom-

panying scientific publications were two reviews, a history of the epidemiology of dengue and DHF/DSS (82), and a memorandum that summarized the meeting, coauthored by 10 participants (83). The conclusions, organized as 16 topics for future research, pose challenges still relevant today (Table 2). It is elating to note that many of these research goals have been met. For example, methods for isolation of DENV or recovery of DENV viral nucleic acid from patients or mosquitoes (topic 1), classification and antigenic analysis of dengue viruses (topic 2), and rapid identification of dengue virus proteins in acute-phase blood (topic 14) are now well established. We are still not completely confident whether precisely the same dengue viruses cause mild and severe disease (topic 3). The mosquito vectors and zoonotic hosts of chikungunya and dengue viruses are reasonably well established (topics 8 to 11). The pathophysiology of many manifestations of human dengue disease have been intensively studied (topics 6 and 7). The call for the development of diagnostic criteria for the several dengue syndromes (topic 15) was provisionally answered in this memorandum and then by a WHO Technical Advisory Committee in 1975, which was subsequently updated in 1986 and 1997 (84–86). Effective mosquito control measures have eluded public health organizations over the past 50 years (topic 13). Dengue vaccine

TABLE 3 Suggested definition and nomenclature for diseases suspected to be of dengue or chikungunya virus etiology^a

Fever and type/definition
Undifferentiated fever
Etiology unknown
Etiology known
Dengue fever (this should include febrile disease characterized by myalgia and/or arthralgia and leukopenia, with or without rash or lymphadenopathy, but including many of the following: biphasic fever, severe headache, pain on moving the eyes, positive tourniquet test, and a fever spontaneous petechiae)
Etiology unknown
Etiology known
Hemorrhagic fever (this includes fever usually without prominent myalgia or arthralgia, positive tourniquet test, and leucopenia or rash present or absent, but usually with several of the following: extensive spontaneous petechiae, purpura, ecchymoses, epistaxis, hematemesis, melena, thrombocytopenia, prolonged bleeding time, and maturation arrest of megakaryocytes)
Without shock
Etiology unknown
Etiology known
With shock. The pulse pressure is 20 mm Hg or less or systolic and diastolic pressures unobtainable, with collapse of the patient. Shock may occur without the hemorrhagic manifestations described above but with most of the following associated with serious disturbances of the hemostatic mechanism as essential criteria if hemorrhagic fever has been diagnosed: positive tourniquet tests, thrombocytopenia, prolonged bleeding time, and maturation arrest of the megakaryocytes.
Etiology unknown
Etiology known

^a Adapted from reference 83 with permission of the publisher.

development has been continuously pursued for these same 50 years (topic 12). Amazingly, we are still searching to understand the precise pathogenic mechanisms of DHF/DSS (topic 4) and to identify the sites of dengue virus infection in human tissues (topic 5). What is our grade for 50 years of effort? Perhaps it is a C-minus?

DENGUE PATHOGENESIS: LOOKING TO THE FUTURE

During the decade of the 1960s, the syndromes DHF and DSS were physiologically, virologically, and serologically defined. An issue in the dengue field that was first approached during that decade continues to be the promulgation of case definitions equally useful to clinicians and epidemiologists. In the 1960s, two case definitions were published. The first was a consensus statement devised at the 1964 WHO meeting (Table 3) (83), and the second was developed by S.B.H. and published in 1970 (see above) (43). Subsequently, case definitions for DF, DHF, and DSS were published by a WHO committee in 1975 and revised in 1986 and 1997 (84–86). The DHF case definition required evidence of hemoconcentration and either a positive tourniquet test or overt bleeding. However, the absolute requirement for observable bleeding and the widespread difficulty encountered in estimating hemoconcentration using microhematocrit values led to the development of new case definitions based upon symptoms and signs (75). It is interesting to speculate if that the authors' 1970 DHF case definition, which did not specify a positive tourniquet test or bleeding signs as absolute requirements but instead listed a low serum albumin value plus altered hemostasis, had been adopted, today's case definition arguments might have been avoided. The revised 2009 WHO case definition (75) does not include altered hemostasis, commonly identified in the 1960s as a prolonged bleeding time, or complement activation, which are well-established components of DHF (66).

During the decade of the 1960s, strong evidence was provided

that DHF/DSS occurred in two different immunological settings: (i) a majority of cases were accompanied by an anamnestic dengue antibody responses (these had been documented in field studies as the result of a second heterotypic DENV infection) and (ii) there was a smaller group of infants with primary antibody responses. The mothers of these infants were shown to be dengue immune. It had been observed that the severity and frequency of second-infection "altered dengue" were impacted by infection sequence and the interval between the first and second infections (43, 87). It was evident then that whatever the immunological phenomenon underlying DHF/DSS, their existence raised important caveats about the development and use of vaccines (59). During the decade, a pathogenesis hypothesis was offered to explain DHF/DSS accompanying a second DENV infection: DHF/DSS might be caused by pathogenic IgG-DENV immune complexes that fixed complement (58, 63, 88). However, the Arthus-type lesions of IgG-mediated hypersensitivity were not observed in tissues from fatal DHF/DSS cases (43). Also, acute immune complex disease failed to explain DHF/DSS in infants. How could infants with low concentrations of passively acquired maternal IgG DENV antibodies at the onset of infection mount a disease-provoking immune response with the same kinetics as children with a secondary immune response? No plausible explanation was available (43).

It is remarkable that so much dengue pathogenesis research effort over the past 45 years has focused on mechanisms that address only sequential DENV and ignore primary DHF/DSS infection in infants. These mechanisms, exaggerated or abnormal T cell responses, and autoimmune responses evoked by DENV antigens are well described in many reviews (89–91). During the decade of the 1970s, several experimental observations led to an explanatory hypothesis that was able to accommodate both groups of cases. DENV 2 viremias were higher in monkeys experiencing a second infection than in those with a primary infection (92). Next it was found that cultured monocytes obtained from DENV-immune

humans or monkeys supported DENV infection *in vitro* (93). Further, mixtures of IgG anti-dengue virus antibodies and DENV, when added to cultured monocytes obtained from nonimmune individuals, resulted in enhanced infections (94). Finally, low concentrations of polyclonal dengue virus antibodies administered to nonimmune rhesus monkeys enhanced DENV 2 viremia (95). Dengue virus antibodies, whether actively raised or passively acquired, could result in enhanced DENV infections (antibody-dependent enhancement [ADE]). Recently, a fatal vascular permeability disease was demonstrated regularly in mice when DENV infections occurred in the presence of enhancing antibody concentrations (96, 97). Many questions intrude on applying ADE as the universal phenomenon causing human disease. Why does DHF/DSS occur in only around 2 to 4% of secondary dengue infections (98)? Why does the acute vascular permeability phenomenon occur so late, just as viremia and fever end and while cellular infection is being cleared (99)? Very recently, a new pathogenesis phenomenon has been discovered that is consistent with ADE being a principal mechanism of pathogenesis. DENV NS1 is a direct toxin (100, 101). Peak DENV NS1 production predicts the severity of DENV infections (102). Now it has been shown that NS1 directly damages endothelial cells *in vivo* (101). It is known that NS1 contributes to activate complement and to altered hemostasis and liver damage (100). Could DHF/DSS be a viral infection equivalent of staphylococcal toxic shock syndrome? Now that the three-dimensional structure of DENV NS1 is available for study, accelerated research may finally explain why and how severe vascular permeability accompanies DENV infections in individuals who are partially dengue immune (103).

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